Bull. Environ. Contam. Toxicol. (1990) 45:1–5


Bulletin of Environmental Contamination and Toxicology

# Sources of Error in the Determination of Trichloroethylene in Blood

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One of the more common methods in determining volatile chemicals in biological samples involves an extraction procedure followed by a chromatographic quantitation step. These analytical methods are generally validated using conventional techniques with some attempts to minimize analyte losses during sample handling (Kroneld 1985, Reunaneu and Kroneld 1982). After a successful validation, the analyst will feel confident that the procedure employed would provide an accurate determination of the analyte in the sample. However, it is difficult or sometimes impossible to identify precisely the losses that occur during sample handling. In this paper problems associated with the accurate determination of the volatile analyte, trichloroethylene, (TCE) are addressed.

## MATERIALS AND METHODS

Gas chromatography was performed with a Perkin-Elmer Sigma 2000 chromatograph equipped with a 63Ni detector, a split/splitless capillary inlet system, a 30 m x 0.32 mm i.d. fused-silica column coated with a 1.0-μm film of 5% phenyl methyl siloxane (DB-5) , and an AS-100 autosampler. The system was used in the split mode (~1:40). The column was operated isothermally at 80°C, with inlet and detector temperatures of 150 and 250°C, respectively. Helium, at a flow rate 1 mL/min, was used as the carrier gas, and argon/methane (10%) was delivered at 30 mL/min as the detector makeup gas. A laboratory data system, Hewlett-Packard model 3359, was used.

Experiments to determine recovery of TCE were performed on both water and blood samples. To obtain basic information in the partitioning of TCE between blood and n-hexane, standard solutions of n-hexane containing both internal standard (chloroform, 0.14 μg/mL) and TCE in the concentration range of 0.014 to 1.40 μg/mL were prepared. Two-milliliter ali-

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quots of these solutions were added to 10-mL vials containing 0.2 mL of control blood. The vials were sealed and vortexed. For the determination of analyte stability, some vials at each concentration were stored up to six days at a temperature of -20°C. The remaining vials were analyzed immediately. On the day of analysis, an aliquot of the hexane layer was removed, placed into an auto sampler vial, and analyzed using the GLC conditions described above.

In the second set of experiments, samples were prepared simulating conditions used in blood-sample collection. Ten-milliliter headspace vials containing stir bars were filled with either water or control blood and sealed. These vials contained no headspace. Ten-microliter aliquots of methanolic TCE standards of various concentrations (14-1400 μg/mL) were injected through the septum into the various vials. The resulting TCE concentrations were identical to those prepared for the first experiment. The samples were maintained at 37° ± 0.5°C in a water bath and stirred for 2 hr. Subsequently the septa were punctured with two 22-gauge needles, one of which was connected to 30 cm of polyethylene tubing. Samples were drawn through the tubing, and 0.2-mL aliquots were taken using a polypropylene tuberculin or glass gas-tight syringe. All aliquots were analyzed following the same procedure used for the first experiment, i.e., adding 2 mL of hexane containing 0.28 μg of chloroform.

## RESULTS AND DISCUSSION

Results of the first experiment, which was a typical validation study for the determination of TCE in whole blood, are shown in Table 1. The validation was conducted by adding aliquots of a solution of TCE in n-hexane to aliquots of control whole blood. Results indicated excellent recovery and high precision. Stability of TCE in blood samples was evaluated by

Table 1. Performance evaluation of blood analysis method for trichloroethylene

| Equivalent Blood Concentration (μg/mL) | Recovery (%) | SD (%) |
|---|---|---|
| 0.14 | 92.0 | 5.1* |
| 0.28 | 93.9 | 4.9* |
| 0.70 | 95.8 | 2.1* |
| 1.40 | 99.1 | 0.6* |
| 2.80 | 99.2 | 0.5** |
| 7.00 | 100.0 | 0.8** |
| 14.00 | 99.4 | 0.9** |
| 28.00 | 98.1 | 0.5** |

*n = 3
**n = 4

adding TCE and chloroform in n-hexane to whole blood and storing the blood samples with the n-hexane extracting solvent at -20°C for up to six days (Table 2). Minimal losses were seen as evidenced by the equivalency of the absolute chromatographic peak areas as well as peak area ratios.

Table 2. Stability of TCE in blood samples

| Day | Concentration (µg/mL) | % of Day 0 | SD* (%) |
|---|---|---|---|
| 0 | 0.014 | 100.0 | 3.2 |
| | 0.140 | 100.0 | 0.3 |
| | 1.400 | 100.0 | 0.2 |
| 1 | 0.014 | 91.8 | 4.9 |
| | 0.140 | 99.0 | 0.8 |
| | 1.400 | 99.6 | 0.7 |
| 6 | 0.014 | 93.4 | 6.3 |
| | 0.140 | 96.7 | 0.8 |

* n = 4

Given these results it might be presumed that accurate analyses of blood samples could be conducted. However, the question of the effects of sample handling on the accuracy of the analysis must be taken into consideration.

To evaluate the effects of sample handling, a second set of experiments were conducted in which a system was devised which mimicked the blood-collection procedure. The results are presented in Figure 1. When water was used as a matrix, and a glass, gas-tight syringe was used for sampling in the system, recovery over the range 0.56 to 1.40 µg/mL of TCE averaged 93 ± 2%. However, when a disposable polypropylene, tuberculin syringe was used, recovery from the fortified water samples dropped to 86 ± 1%. When the experiment was repeated with the tuberculin syringe, with whole blood as the matrix, the recovery was slightly lower at 83 ± 1%. The precision of the analyses was very good. The additional losses found with the tuberculin syringe could be due to head-space losses or adsorptive losses on the polypropylene (Barcelona et al. 1985). However, the endogenous lipids in blood should preclude major adsorptive losses. Since TCE recovery from whole blood using a tuberculin syringe was very similar to recovery from water, the more likely explanation is that head-space losses are occurring. Unfortunately, the routine use of glass, gas-tight syringes for sample handling would not be economical. It can be concluded that losses of approximately 15% can be expected when blood samples are drawn from the subject.

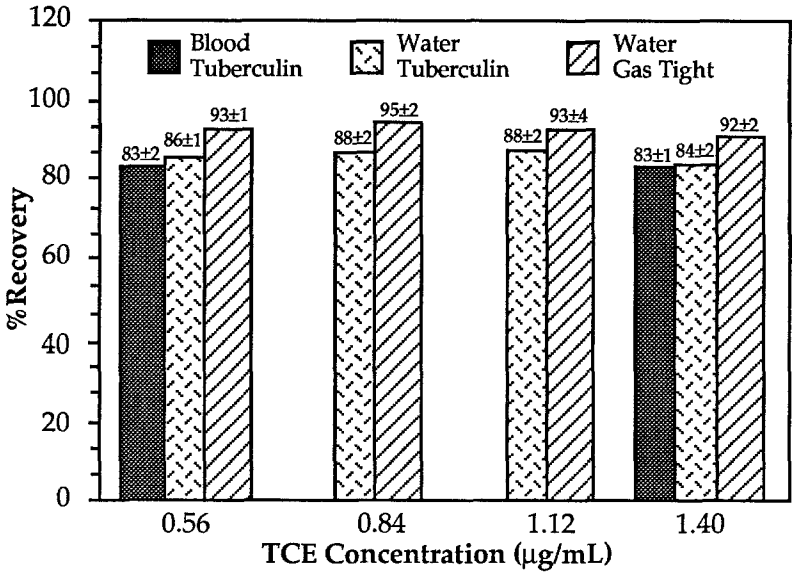


Figure 1. Recovery of TCE from whole blood or water samples obtained using tuberculin or gas tight syringes. (n=6)

In most studies the concentration of analyte is determined in serum or plasma, not whole blood. In order to evaluate the losses incurred during the centrifugation step to separate red blood cells from serum, whole blood was drawn with a tuberculin syringe from the heated 10 mL vial, as described in the materials and methods section, and centrifuged.

Both the red blood cell (RBC) and the serum fractions were analyzed for TCE (Figure 2). In the rat the RBC to serum volume ratio is approximately 0.5. It was determined that there was approximately a 50% decrease in the concentration of the TCE in serum compared to the experimentally determined concentration in whole blood. On the other hand, the concentration of TCE in red blood cells was found to be approximately 20% higher than in whole blood. It is concluded that some concentrating occurred in the red blood cells but that an additional 30% of the TCE in whole blood was lost during the separating process to obtain the serum.

To further evaluate the factors involved in the accurate determination of TCE in blood, the serum was kept frozen overnight and extracted and analyzed the following day. It was found that the concentration dropped by almost 50% compared to the fresh serum. The final concentration determined in the serum stored overnight was approximately 20% of the initial concentration determined in whole blood.

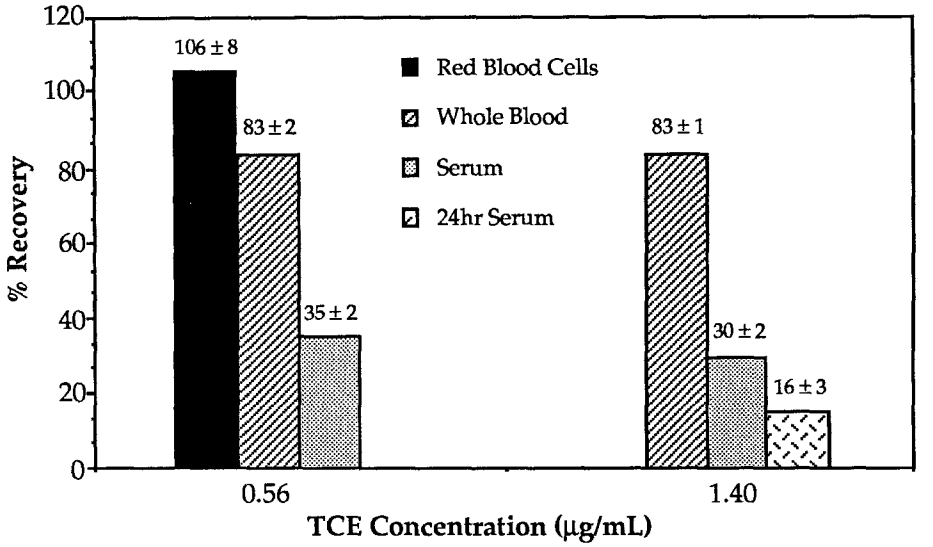


Figure 2. Recovery of TCE from whole blood or water samples obtained using tuberculin or gas tight syringes. (n=6)

From our experience with the volatile analyte TCE, it is obvious that extreme caution and a well defined protocol are needed to achieve accurate analysis of volatile chemicals in biological samples. The recommended procedure is to use whole blood, to draw the sample slowly to reduce headspace in the syringe and to minimize sample handling by transferring the blood sample directly to a vial containing the extraction solvent immediately after it is drawn. Blood samples should be analyzed within 24 hours after collection

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Received November 11, 1989; accepted January 28, 1990.